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Agar in Australia

(Division of Fisheries—Report No. 12)

By

E. J. FERGUSON WOOD, B.A., M.Sc.

MELBOURNE, 1946

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FOREWORD.

Until the outbreak of war with Japan, that country was the only one which had systematically studied its seaweeds and their possible exploitation for human needs. In some other countries seaweed was used as a food to a limited extent; brown seaweeds had been tried as a fodder, and had been treated to yield iodine, potash, and latterly, alginates; agar was made from some of the red seaweeds in California and Russia. The main source of agar was Japan, and this commodity was the one seaweed product of wide commercial importance in Allied countries. Its use for medical purposes is the most important, but agar has no adequate substitute for many things that would have been considered necessary in peace-time, e.g. in canned meats. Nowadays the use of agar for any purpose other than bacteriology is prohibited in Britain and the United States, and this is a measure of the scarcity of the product. Australia is now in a position to produce agar for meat canning, and should soon be in a position to supply bacteriological agar for overseas use. In the meantime, New Zealand and America are producing bacteriological agar, though the demand is not yet satisfied. In this country, lack of the necessary freezing equipment has been the chief reason for the delay in producing high grade agar, although the high setting point of agar from *Gracilaria confervoides* has been somewhat discouraging.

The present paper deals with agar production in a comprehensive manner, and is an attempt to recapitulate as briefly as possible the work that has been done on agar in Australia and elsewhere. A separate paper discussing the raw materials in greater detail and designed for those engaged in harvesting the seaweed is now in press.

It is hoped that the foundation of an agar industry in Australia and its obvious importance to this country, especially in time of war, will continue to stimulate algological research here, for agar is only one of a possible number of seaweed products which need investigation.

Agar in Australia *

I. INTRODUCTION.

The substance "agar-agar" or "kanten" has many commercial uses, and has been essential for certain purposes intimately connected with the war. Until the outbreak of war in 1939 only a single attempt had been made to produce agar-agar in Australia. This was in Western Australia in the last century. The project was not developed.

The name "agar-agar" is applied to certain common edible red algae in Malaya, and "kanten" is Japanese for "cold sky", because frost is necessary for its preparation. There is a tendency nowadays to refer to the substance as "agar" and this will be followed here. Before the war, Japan supplied nearly 95 per cent. of the world's agar, though China produced some agar for her own consumption at Ningpo, Tsingtao, and Chefoo (64), and some was also prepared in Malaya. Latterly America produced as much as 50,000 lb. in a year in California, though the quantity has varied very considerably. Russia was also entering into the production of agar and agaroids.

Australia's entire imports of agar came from Japan, and, as Japan was a non-sterling country, supplies were seriously reduced in 1939, and were stopped by the economic sanctions of 1941. The imports to the United States and Britain were stopped by Japan's entry into the war, and the agar position in the United Nations has, since then, caused some concern.

The gap has been filled to some extent by an increase of production in California, and by manufacture of agar in Australia and New Zealand. The Fisheries Division of C.S.I.R. became interested in agar prior to Japan's entry into the war, and the writer carried out some research.

No information was available on the distribution in Australian waters of red algae suitable for agar production. The only material was in herbarium collections which had been incompletely classified. There were no distribution studies nor quantitative estimates of abundance of such seaweeds. It was known that many fishermen's wives living on the south-east coast of Tasmania used one of the red algae, *Gracilaria* sp., for thickening jams and for making jellies, but there were no reports of regular or large occurrences, and it is still doubtful whether there is sufficient raw material for commercial agar production in Tasmania. In Western Australia, *Eucheuma speciosum* and *Pterocladia lucida* were used by housewives for jellies.

The discovery of a quantity of seaweed on the beach at Bateman's Bay, N.S.W. by Mr. R.H. Pilmer, and his

* Typescript received October 19, 1945.

recognition of it as one sold by the Chinese for thickening soups, altered the position. The seaweed was identified by Associate-Professor Ethel McLennan of the Botany Department, Melbourne University, as *Gracilaria confervoides* (Linn.) Grev. Mr. Pilmer later got in touch with Mr. Laver of the State Fisheries Department who reported the occurrence to the Fisheries Division. Some agar was forthwith prepared and was found to be generally comparable with Japanese agar.

Mr. R. Bouchier of Cronulla recognised the seaweed when shown it by the writer, and brought in a sample from Port Hacking in March, 1941. In June, Mr. Bouchier, and officers of this Division found large quantities of *Gracilaria confervoides* at Kurnell in Botany Bay. This beach occurrence showed that there was a reasonable quantity of the raw material for agar production in New South Wales, and experiments were carried out by the writer which showed that good quality agar could be produced from it.

A great deal of experimental work has been done on methods of manufacture, and a field programme aimed at studying the growth and occurrence of suitable seaweeds has been carried out, while further field work is continuing. Taxonomic studies on the red algae are in progress. It is the purpose of this report to survey the agar position, and to discuss the work done in connection with agar production, especially in Australia.

II. PRODUCTION AND DEMAND.

Information on the production of agar and on the demand for it is not abundant, what exists being rather vague. As far as can be learned, the following figures in short tons give a reasonable picture of the pre-war position. They have been collected from various sources.

TABLE 1 - Figures for Production and Use of Agar.

	Production	Use
	short tons	short tons
Japan	c.2800	850
Russia	55	
	+ 200 tons of agaroid	500
America	0.5-22	300
Britain	0	200
China	38? 75?	175
Malaya	?	?
Germany	0	?
Australia	0	71
New Zealand	0	18

At the present time, America is aiming at a production of 50-75 tons per annum - her annual need for bacteriology and medical purposes. New Zealand aims at 15 tons, and Australia at 60 to 100 tons, although the present production is of the order of 2 tons per month. Present information regarding the supply of seaweed suggests that at least 100 tons can be produced annually in Australia from known sources. These lie south of a line from Rockhampton in Queensland to Northampton in Western Australia. Little is known of the coast north of this, although an agar-bearing weed is known to occur near Cairns, and *Gracilaria* has been reported in quantity north of Shark Bay. The last report has not been confirmed.

III. THE USES OF AGAR.

1. General.

Agar is used on account of the peculiar properties of its gel, especially its marked stability, high viscosity and hysteresis, its swelling with water, and its stabilizing effect on emulsions.

The commercial demand for agar is greater than the supply, even in normal times, owing to the increasing use of stabilizing agents in emulsions, and to the popularity of jellied food packs. The commercial uses include meat and fish preserving, confectionery, spices, condiments, vegetable pastes, aerated waters and fruit juice cordials, water-paint manufacture, vinegar and wine manufacture, leather and cotton dressings, etc. Agar and agaroids are used as stabilizers in ice-cream and other commodities.

Scientific uses are of great importance in the war effort, but require only a relatively small quantity of agar. They include bacteriological media (diagnosis, preparation of vaccines etc.), dental impression materials, laxatives, surgical dressings, photographic film, and chemical and physical uses (pH determinations, studies in diffusion, electrochemistry, and research on the properties of gels and sols).

The following table supplied by courtesy of the Department of Supply and Shipping gives the industrial uses of agar in Australia in 1938.

TABLE 2 - Uses of Agar in Australia.

Industry	lb. per annum
Meat preserving	99,350
Confectionery	33,235
Spices and condiments	24,416
Chemical, medical, etc.	1,459
Jams and vegetable pastes	1,459
Aerated waters and cordials	336
	160,255

It is interesting to quote Tseng's figures (64) for the uses of agar in the United States. They will be very suggestive to agar manufacturers in Australia wishing to increase the uses of agar after the war.

TABLE 3 - Uses of Agar in the United States.

Industry	lb. per annum
Laxative	100,000
Microbiology	100,000
Bread, etc.	100,000
Confectionery	100,000
Dental impression material	75,000
Meat packing	50,000
Emulsification	50,000
Cosmetics	25,000
Miscellaneous	50,000
	650,000

Agar is used to improve the keeping qualities of bread in America and Russia, and this development may be expected in Australia. One may expect, too, an increased use of agar in meat packing, and its possible extension to canned fruits.

2. Meat Preserving.

Agar is used in such commodities as canned tongues, camp pie, galantines, canned rabbit, canned mutton bird, and in other foods where appearance is improved by a firm white jelly, or where it is desirable to remove excess moisture from the meat while retaining it in the can. In canning practice, it is important to have a substance which sets after being heated to the maximum sterilizing temperatures used. To ensure killing all the bacteria in meat and fish products, the centre of the can must reach the sterilizing temperature and remain there for about half an hour. This means heating the surface of the meat to the vicinity of 230°-240° Fahr. (7-10 lb. pressure) for periods of up to 1½ hours. At such temperatures and pressures, gelatine tends to hydrolyse to give proteoses and peptones, and loses its power to set again on cooling. Agar, on the other hand, is not hydrolysed appreciably under 2 hours at 26 lb. (266°F. or 130°C.) and sets to a firm gel again on cooling. Moreover, gelatine melts and remains liquid in the vicinity of 77°F. (25°C.) while agar sets between 87 and 122°F. (30 and 50°C.) and does not melt until 176-203°F. (80-95°C.) is reached. In the tropics, where temperatures of 90°F. (32°C.) are usual, agar is much preferable to gelatine even in cans processed at low temperatures. Moreover, agar is stable to a pH of 4.5, at boiling point and lower, in the cold, while gelatine tends to break down below pH 6. This is important in packing acid foodstuffs, e.g. fruit juices.

3. Confectionery.

Agar is used to produce the "short" gel necessary for certain sweetmeats, and also as a stabilizer. It does not hydrolyse with age as gelatine tends to do. Agar, sodium alginate, and gelatine are all used as stabilizers in ice-cream.

4. Spices and Condiments.

Agar is used as a stabilizer in mayonnaise, and in vegetable pastes where its water absorptive action is also needed.

5. Other Commercial Uses.

In water paint it acts as an emulsifier, while in the wine and vinegar industries it acts as a clearing agent. Certain firms in Sydney are using the seaweed *Gracilaria*, preparing their own clearing agent from it. In some Sydney tanneries also, the raw seaweed is bought and used for glazing upper leather. In the textile industry, agar is used as a size, and in the paper industry it is used for waterproofing, and for duplicator rolls. Some greaseless cosmetics have an agar base, and so have some dentifrices, though it is suggested that some of the agaroids (see below), e.g. that of *Cataneella* sp. or *Grateloupia* sp., might be better for this purpose. Agar is also used in welding fluxes and in the drawing of tungsten lamp filaments.

Sharp (58) suggested the use of agar as a dispersion medium for nicotine sprays, and found that karaya gum and agar give these sprays three times the normal lethal effect. He used 1 part agar to 500 by weight. Agar was superior to karaya gum as there was no precipitate.

6. Bacteriological Media.

Agar was first used as a base for bacteriological media by Dr. Walter Hesse on the suggestion of his wife, Frau Fanny Hesse (19). It was found that gelatine, which had been used previously, had three drawbacks:-

- (i) It is difficult to sterilize, owing to its hydrolysis at sterilizing temperatures.
- (ii) It cannot be incubated at 37°C. (98°F.), the optimum temperature of most human pathogenic bacteria.
- (iii) It is liquefied by a number of common bacteria including many of those occurring in the human intestine.

Agar has none of these drawbacks. It is certainly liquefied by a few bacteria of marine or soil origin, but these organisms have an optimum growth temperature below 25°C. (78°F.) and are not pathogenic to man. Silica gels are displacing agar for some bacteriological uses, but these must be prepared first and the bacterial inoculum placed on the surface, whereas agar can be mixed with the bacterial suspensions. The general conclusion is that for many purposes, e.g. in medical and in water and sewage bacteriology agar is essential. Latterly pectin and agaroids (e.g. Irish moss) have been tried with only limited success. Even when carrageen is assisted by 0.75 per cent. agar, the medium is inferior to agar.

In bacteriology, agar is added to nutrient solutions to give a solid medium which is relatively transparent, and on the surface of which bacteria will grow. If bacteria are sown into an agar medium they will produce colonies which are held stationary by the agar, and can be counted. This is the principle used in "plate counts" or "viable counts". Vaccines such as T.A.B. (Typhoid, Paratyphoid A & B),

cholera, gonococcal etc. are prepared by growing cultures of the required organisms on agar surfaces with suitable nutrients, washing off and formalizing the growth. Agar is preferable to broth for this purpose because the growth on agar is on the surface and little food material is washed off with the growth. This is desirable on account of the possibility of protein reaction (anaphylaxis) in the patient from the nutrients in the medium. Again, agar is used for counting bacteria in milk, water, sewage etc., and as a substrate in the diagnosis of scarlet fever, meningitis, and many other diseases. Agar also possesses certain growth factors which promote the growth of organisms (26,50). It is probably for that reason that Itano and Tsugi (23) find that *Asotobacter chrooococcum*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* grow better on lower grade agar. Jensen (26) and the writer found that growth substances in commercial *Gracillaria* agar assisted the growth of many bacteria but that these substances plus the nutritive material were a disadvantage in counting bacteria on poured plates. These growth factors were reduced by purification. The agar obtained from *Gracillaria* sp. from Tasmania has, as at present manufactured, too high a viscosity to be of use for bacteriology, and the same applies so far to agar produced from *Eucheuma* spp. The agar from *Gracillaria confervoides* is suitable if the viscosity is lowered by careful preparation, though the setting point remains awkwardly high for some purposes. *Hypnea musciformis* gives an excellent bacteriological agar, but, though widespread in New South Wales estuaries, is nowhere abundant enough to warrant special collection. It has a very low viscosity, and a setting point just about 30°C. (86°F.). Tests on these agars have been carried out by Jensen at the request of the writer, and considerable work has also been done by the writer on marine and fish-spoilage bacteria, which confirms Jensen's findings (private communications).

7. Dental Impression Materials.

A number of patents exist for dental impression materials using agar as a base, and it is now regarded as essential for this purpose (43). In this case, the hysteresis (lag between liquefying and setting temperatures) is used. The concentrated agar solution is heated to liquefy, placed in the cavity, and allowed to set. A low setting-point agar is preferable for this purpose.

8. Laxatives and Surgical Dressings.

Many laxatives consist of liquid paraffin as a base, with agar, benzoic acid or lactic acid, and possibly gum acacia or gum tragacanth. Although in some cases agar is used as a laxative *per se*, it is usually employed in small amounts of the order of 0.65-0.75 per cent. (44) and is therefore an emulsifying agent. Only one standard laxative used in this country has in its composition enough agar for that substance to exert its absorptive effect. Dry chopped agar is sometimes used in a 10-gram dose. It appears to be without effect on the digestive functions (44) and hastens peristaltic action by an increase of volume, i.e. swelling. Powdered agar is stated to have an irritating action on the mucous linings of the alimentary canal, though this is not the case with flake or strip agar.

Agar is used in surgical dressings on account of its anti-coagulant properties, and also in greaseless dressings.

9. Chemical and Physical Uses.

These include the use of agar for bridges in determining the e.m.f. of cells where diffusion is required without gross mixing, for diffusion experiments, electrochemical tests, and researches into the structure of gels and sols.

10. Dietetic Aspects.

From the dietetic point of view, agar, in the concentrations used commercially in foods, has little value except perhaps for certain growth factors, and possibly for some trace elements, e.g. iodine. Studies on the polysaccharide (non-nitrogenous) extract by Nilson and Schaller (42) show that 10 per cent. concentrations gave a 20 per cent. increase in weight in rats but required about the same amount of food per gram gain in weight. These authors suggest that the 10 per cent. concentrations had a beneficial effect on the functioning of the intestinal tract (laxative effect).

IV. THE PROPERTIES OF AGAR.

In the discussion of the uses of agar a number of properties have been mentioned, and, in a study of the manufacture of agar which follows, some other properties will assume importance. The chief properties mentioned are: gelation, solution, swelling, hysteresis, stabilizing effect. Those of most importance in manufacture are viscosity, temperature of solution and gelation, syneresis, effect of electrolytes and dehydrating agents, pH effects, and lastly the chemical composition and structure.

Tseng (64) has defined agar as "The dried amorphous gelatine-like non-nitrogenous extract from *Gelidium* and other red algae, being the sulphuric ester of a linear galactan, insoluble in cold but soluble in hot water, a dilute neutral solution (1-2 per cent.) of which sets upon cooling to a firm gel solidifying at 35-50°C. and melting at 90-100°C."

This definition fits the commercial conception of agar very well, but has some disadvantages. The writer suggests a definition on the following lines: "Agar is derived from certain members of the Rhodophyceae and consists of the sulphuric ester of a galactan complex, which, with water, forms a lyophilic colloidal solution liquefying between 70° and 100°C. and setting between 30° and 50°C."

Agar is distinct from gelatine in a number of its properties including its chemical composition - agar is a polysaccharide and gelatine is a protein. Agar swells in cold water taking up a quantity of the solvent and cannot therefore be said to be insoluble like an inorganic compound such as silica. The above definition would include some of the agaroids which have a similar chemical composition but give a mucilaginous rather than a solid gel. It is difficult to separate these as the writer has found a series with intermediate properties, and, in some cases at any rate, the method of preparation alters the properties from those of an agar to those of an agaroid.

Most users of agar can define their requirements in terms of viscosity and setting point, but are vague in their definition of gel strength. Some take as their measure the degree of distortion produced by a given weight, while others measure the gel strength by the weight required to force a given penetrometer through the gel. It appears best to consider gel strength as the breaking strain of a gel, and to measure distortion in terms of elasticity. Thus we can readily distinguish between a brittle gel (of low elasticity) and an elastic gel, and between a strong and a weak gel. *Catanelia* gives a fairly weak but highly elastic gel, while *Gracilaria confervoides* and *Eucheuma* give strong but inelastic gels, and *Gracilaria lucasii* gives a weak and brittle gel.

1. Physical Properties.

It is now necessary to consider the properties of agar from a physico-chemical point of view. As has been stated above, agar is a lyophilic colloid. That is, it does not dissolve in water to yield positive and negative ions as does, say, salt, but becomes dispersed in the water, each particle becoming charged, in this case negatively. If agar is placed in cold water, it will swell and absorb a large quantity of the water, in which case the water is dispersed in the agar, that is, the water constitutes the disperse phase while the agar is the continuous phase. On heating with excess water at about 90°C. the agar becomes the disperse phase and the water the continuous phase. We then get a sol. As the sol cools we get the formation of a gel, at which time there is a change in the condition of the particles. The water absorbed in the formation of the sol is not extruded again in gelation although there is an exudation during extrusion (syneresis - see below).

There are a number of theories to account for these phenomena. Probably the most attractive is that of Krishnamurti (33). Colloids of the agar type are usually regarded as consisting of molecules (in this case galactan molecules) united to form micelles, the unit particles of the dispersed colloid. Krishnamurti believes that an agar sol consists of agar micelles having between them a solution of agar. "Lowering the temperature of the sol to about 35°C. brings about a supersaturation of the molecularly dispersed agar which therefore tends to precipitate. In the earlier stages it is probable that fresh colloidal particles are formed, but, when these are sufficiently numerous, further supersaturation is relieved by the agar condensing on the particles already in the sol. Each of these micelles is surrounded by a hydration layer, and the union of these hydrated micelles brings about the formation of a gel." Bungenberg de Jong (7) believes that gelation occurs first by a clumping of the particles of micelles and then by a further aggregation due to a redistribution of the charges on the surface of the micelles. Studies of the scattering of light from the agar gels (Tyndall effect) by Hatschek (17) and Krishnamurti seem to support Krishnamurti's theory. We have in the agar-water system, agar particles intimately associated with water of hydration, and the water (plus agar in true solution) acting as a dispersion medium (intermicellar liquid). Such colloids in which hydration occurs are known as hydrophilic colloids. In the hydrophobic colloids, hydration does not occur. In both types, the particles are electrically charged. Lyophobic colloids (including hydrophobic) are flocculated by discharging them, but lyophilic (including hydrophilic) are not. To precipitate

these it is necessary to dehydrate them as well. Thus salt will not flocculate an agar sol, but sodium sulphate, which is also a dehydrating agent, will. Pure alcohol will not flocculate agar, but alcohol containing a trace of electrolyte will do so. The swelling of agar in cold water is a hydration effect, while the freezing of an agar gel results in the removal of intermicellar water but not of the water of hydration. The latter is much more difficult to remove. It will be noted that agar in water behaves as a lyophilic colloid, but in alcohol it behaves as a lyophobic colloid, as is pointed out by Kruyt (34).

Syneresis is the property of extruding water possessed by gels. It is considered that this is due to a decrease in the surface area of the internal phase, the cause of which is not known. This decrease, which is regarded by Lipatov and Morozov (36,37) as a continuation of the process of gelation, causes some of the intermicellar water to be removed. Freezing of a gel and the consequent extrusion of ice crystals may be regarded as an extreme case of syneresis. Rossi and Marescotti (54) point out that the salt content of the gel is diminished and that of the syneresis liquid increased during the process. This explains the action of freezing in the purification of agar (see below). Increase of pressure or decrease of temperature increases syneresis.

Hysteresis is the lagging between the temperature of liquefaction and that of gelation. Agar shows this phenomenon markedly; a 1.5 per cent. gel will set at approximately 43°C. but under usual conditions will not liquefy until it has been kept for some time at 98°C. Bungenberg de Jong (6) shows that a 1.7 per cent. sol kept at 50°C. and then allowed to gel at 33°C. did not return to its former viscosity when raised to and held at 50°C. within the time of his experiment. Banerji and Ghosh (2) showed, however, that agar gels will melt and set at the same temperature if sufficient time is allowed, though this will be a matter of days. Ghosh (13) states that hysteresis in a given time interval rises with concentration, then falls and then rises again. It is claimed that agar treated with 10 per cent. sodium chloride, washed, and dried at 60°C. liquefies at 51°C. and sets at 42°C. Thus, hysteresis depends on time, is reduced by electrolytes, and varies though not uniformly with concentration. Sowing with a preformed gel or stirring the sol will reduce hysteresis.

The theory put forward by Krishnamurti that the intermicellar liquid is a solution of agar finds support in the work of Lipatov and Morozov (36,37). These authors show the existence of an insoluble or "highly associated" fraction and a soluble or "poorly associated" fraction of agar, and show that the latter exercises a stabilizing effect on the insoluble fraction towards dehydration and gelation. Gelation and the swelling are stated to reside in the highly associated or insoluble fraction. The poorly associated or soluble fraction also causes a lowering of the structural viscosity of the highly associated fraction and a diminution of the total volume of disperse phase. Thus after a certain concentration of agar in water is reached during the swelling of agar in cold water, the soluble fraction begins to exert a counter action against the osmotic swelling strength of the insoluble fraction by decreasing the osmotically bound water; the osmotic pressure no longer increases with increased concentration, but its rate of increase is diminished. In the case of gelatine, the osmotic pressure

(pressure of swelling) increases strongly with concentration, but with agar the increase is slow. The temperature coefficient of the osmotic pressure of gelatine is also much greater than that of agar. This means that the intramolecular attraction is much greater in agar than in gelatine as Lipatov and Morozov point out. The relative intramolecular attraction of the two substances is shown by the fact that agar must be heated to about 100°C. to produce a sol, while gelatine easily gives a sol at 35°C. i.e. hysteresis is a function of intramolecular attraction.

As will be shown later, *Gracilaria confervoides*, the source of agar in Eastern Australia, gives an agar of much higher gel strength if it is not washed prior to extraction than if it is washed for 24 hours either in running water or in changes of water. This is believed by the writer to be due to the removal of some at least of the soluble fraction of the agar. The fact that gel strength is increased by the freezing and thawing of agar is attributed to syneresis, in which both fractions are retained in the gel and only impurities escape with the water removed. A change in aggregation may also occur. This is suggested by the fact that flocculation with alcohol or an electrolyte has a purifying effect but does not give the increased gel strength which is produced by freezing.

So far we have been considering agar in terms of its "solid" or gel state. For practical purposes however it is also necessary to consider it as a "liquid" or sol. The temperature for change of state varies according to the raw material used in manufacture, the concentration, and also varies with time etc. It seems to be independent of viscosity. Within limits the temperature depends on the seaweed used. Thus, no method has yet been found to reduce the setting temperature of *Gracilaria confervoides* agar. This is 40°-41°C.; *Gracilaria* sp. probably *furcellata* about 32°C.; *Eucheuma* agar about 32°C.; whereas *Gelidium corneum* and *G. cartilagineum* agars set at about 30°C., *Pterocladia lucida* agar at about 35-37°C., and *Hypnea musciformis* agar sets at about 28°C. Variations within limits are due to concentration, time, and the previous history of the gel. This makes it difficult to determine the actual setting point of a given gel. The same thing applies also to the point of liquefaction, but this, as stated above, is much more dependent on factors other than the source of agar. Pressure raises the setting temperature, which means in practice that the use of pressure instead of suction in filtering agar sols will cause difficulties and, in fact, has proved the greatest obstacle in translating experiments from the laboratory to the commercial scale. The writer has found a "syneresis" effect whereby the gel on the pressure side becomes more concentrated than that on the other side of a pressure filter.

The viscosity of agar sols, which is the most important factor in their study, increases with decrease in temperature but not linearly. For a range of fairly high temperatures, the viscosity changes slowly, but as the sol approaches the setting point the viscosity increases rapidly until gelation occurs. It is possible to get a false setting point by plotting a cooling curve against viscosity, as time is required for setting once the temperature is reached. Another factor affecting the viscosity of agar gels is their previous history. Continued auto-

claving or heating will affect the viscosity. This is probably due in part at least to hydrolysis. The purity of the agar also affects the viscosity. Jensen (26) found that *Gracilaria confervoides* agar sols became less viscous when the crude agar had been macerated by bacteria, but that the setting point was not lowered. The writer has confirmed this.

Routine viscosity tests made on all samples of agar prepared in this laboratory (approximately 300 tests) have shown for *Gracilaria* agar that viscosity is not affected by:-

1. The method used in drying the weed (artificial drying or atmospheric drying).
2. Bleaching of the weed by chlorine and its removal by sodium thiosulphate.
3. The concentration of weed used in the extraction of agar.
4. Digestion of the raw weed before extraction by papain, pepsin, or bacterial digests.
5. Small admixtures of other agar-producing seaweeds (of the order of 0.65 per cent.).
6. Time of boiling within limits - 15 minutes to 4 hours and in one case to 6 hours.
7. Purification by activated carbon.
8. Purification by alcohol (see p. 31).
9. Temperature of thawing (20°C. - 40°C.).
10. Alcoholic precipitation of agar (see p. 28).

Viscosity of *Gracilaria* agar is lowered by boiling on the acid side of neutrality. This has been confirmed in a number of tests. It is not affected however by alteration of pH after boiling, i.e. before and after filtration. The viscosity is, for some unknown reason, raised by mincing the weed irrespective of the time of boiling.

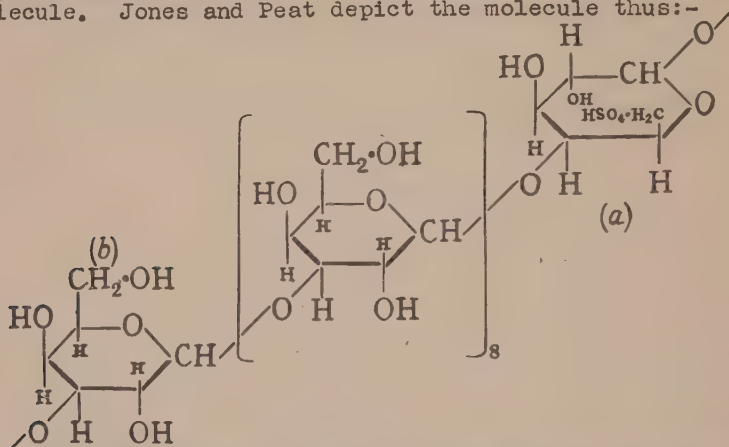
The viscosity is probably increased by the use of pressure extraction (digestion). It possibly varies with the locality and time of year but as the work has been done on bulk samples containing weed in varying stages of maturity, further work is necessary. On the whole, for example, Botany Bay weed has given a higher viscosity than weed from Middle Harbour.

It is intended to include actual figures in a future publication.

2. Chemical Properties.

Chemically speaking, agar is essentially a galactan, i.e. is capable of hydrolysis to the sugar galactose. Neuberg and Ohle (40) found that sulphuretted hydrogen was produced by micro-organisms growing on agar media lacking added sulphur, and concluded that sulphur was present in the agar molecule. They suggested that it contained a sulphate ester linkage, and Haas (15) found the same in *Chondrus crispus* (carrageen). Later work has confirmed this conclusion (57). From a study of the published work on this aspect of the chemistry of agar it would appear that the highly associated fraction of Lipatov and Morozov contains less sulphur than the poorly associated fraction (Neuberg and Schweitzer, 41; Samec and Isajevic, 57). It is considered that, in the plant, agar exists as the calcium salt.

The detailed structure of agar has been investigated by Pirie (51), who suggested the presence of a laevo-galactoside, by Hands and Peat (16), by Percival and co-workers (46,47,48,49,50), and lastly by Jones and Peat (27) who have presented a formula for agar which appears to allow for all the chemical properties of the substances. The last-mentioned authors consider that agar consists of nine dextro-galactose residues mutually combined by 1.3 glucosidic linkages and terminated at the reducing end by a laevo-galactose residue (a) united with the rest of the chain by a glucoside linkage at C₄ and not C₃. This is esterified at C₄ with sulphuric acid. This formula accounts for the isolation of laevo-galactose from agar, the separation of 2.4 di-methyl 3.6 anhydro-laevo-galactose, and 2.5 di-methyl 3.6 anhydro-laevo-galactonic acid from methylated agar, and for the absence of sulphuric acid residues from acetylated and methylated agar. The units are considered as being arranged in chains, which also explains satisfactorily the anisometric nature of the molecule. Jones and Peat depict the molecule thus:-



The difference in properties between the soluble and insoluble fractions of agar is doubtless one of aggregation, and not of chemical composition. The closest analogues to agar to be found in nature are, according to Percival (50), damson gum and the galactogen from *Helix pomatia*, where similar linkages occur.

As has been stated above, agar occurs in the plant as a calcium salt. Because the agar is usually extracted in acid solution, the free acid is normally obtained; frequently, however, the agar acid is neutralized after extraction by lime or soda giving salts of the agar acid. It has been stated (8) that the calcium can be replaced by potassium, sodium, aniline, and hydrogen, and the sulphate by chloride or nitrate without destroying the property of gel formation provided the pH is greater than 2. The rigidity of the gels is increased by the addition of small quantities of alcohol, glycerol, sucrose, and glucose, and by acids provided the pH is greater than 2 (67). At pH 2 hydrolysis occurs at room temperature, but the writer has found that gelation ceases at pH 4 at 95°C. Strong alkali also causes agar to hydrolyse. The agar molecule suffers hydrolysis by prolonged heating at 95°C., and fails to gel after heating for 2 hours at 260°F., but it can be heated to these temperatures for shorter periods without appreciable loss of gelling properties and it often receives such treatment in the canning industry. Agar warmed with nitric acid and neutralized with ammonia gives mucic acid, and

this reaction is often used for estimation. Lüdtke (38) found this reaction unreliable, and the writer, as a result of numerous tests, concurs with this view.

Commercial agar contains a number of impurities, partly on account of the method of preparation and partly because gels are very difficult to purify. Analyses of Australian and Japanese agar are given in the following table:-

TABLE 4 - Partial Analysis of Agar.

	Australian	Japanese
	%	%
Moisture	20.0	21.9
Ash	4.6	3.6
Silica	0.40	0.13
Nitrogen	0.31	0.20
Sulphur	0.37	0.50

In addition to these substances, there are numerous reports of traces of elements in agar. Early in the history of agar research, Leroide and Tassidy (35) reported a trace of arsenic in agar. Fellers, no doubt correctly, ascribes it to the presence of arsenic in the sulphur dioxide used for bleaching. The writer found the same explanation for the occurrence of 35 p.p.m. of lead in early samples of commercial Australian agar. McKinnon records arsenic (39) and arsenic has also been found in Australian agar bleached with sulphur dioxide. Boric acid is recorded by McKinnon and by Kariyone and Terasaki (28), and McKinnon also records iron, aluminium, manganese, phosphoric acid, and carbonate. Iodine is not recorded by McKinnon, but it is noted by Fellers, and estimated by Itano (22) at 24.5-101.9 parts per million. Itano concludes that the iodine content of agar depends largely on the original iodine content of the seaweed, and that it is increased by the use of sulphuric acid or sulphur dioxide for bleaching. Zinc is recorded by Bertrand and Benzon (3) as present at the rate of 6 p.p.m. total weight and 7.7 p.p.m. dry weight. Probably all these trace elements are present as impurities, but with such a complex molecule as that of agar, it is always possible that one or more of them may be essential.

While Japan had a virtual monopoly of the agar industry, the term agar denoted a substance of fairly constant properties, but now that agars are being produced in several countries from different seaweeds it will be necessary to consider the essential differences between them. Agar in this sense now becomes a generic term for a group of closely related components of the cell-walls of the red algae. The Russians were the first to point out the differences between "agar" from various sources, and the work in this country on agar-producing seaweeds has shown that it is necessary to define the term agar more exactly. The chemical composition of the molecule probably remains constant and all "agars" appear to gel in dilute (say 0.5-2 per cent.) solution at temperatures between 30° and 50°C. and to liquefy between 70° and 95°C. under normal conditions (see above). They differ considerably, however, in their viscosity, gel strength, and elasticity. For this reason it is difficult to make a clear division between agars which have a relatively high gel strength and agaroids which have a much

lower one, although the distinction is very useful. Broadly speaking, gels of the *Gracilaria confervoides* type would be classed as agars and those of the *Catenella* type as agaroids (see definition on p. 11).

V. METHODS OF TESTING AGAR.

1. Gel Strength.

After testing a number of suggested instruments for measuring gel strength, a penetrometer was devised which works satisfactorily. The study of a large number of seaweeds required an instrument that would measure a very wide range of gels. Finally, a blunt penetrometer (Plate 1, Fig. 1) was used. It consisted of a glass vessel drawn out at the base to a blunt point, and suspended from one arm of a balance. The gel is placed on a bridge below the penetrometer and the latter is counterpoised. The point is brought in contact with the gel and mercury is run in from a burette until the gel just breaks. The weight of mercury run in is taken as the gel strength. The unit is an arbitrary one, but such penetrometers can be calibrated against each other. The results are very consistent, provided that the gel is carefully prepared. For these tests 2 per cent. of agar is allowed to swell overnight in distilled water, autoclaved at 15 lb. for 20 minutes, poured into dishes and allowed to set for 24 hours. The time factor is not allowed for in this instrument but efforts are made to keep the time of addition of weight as constant as possible.

2. Viscosity.

The measurement of viscosities is, for practical purposes, confined to that of relative viscosities, and for this purpose two types of viscometer exist. The first, typified by the Ostwald viscometer, is standardized against a liquid of known viscosity. The second, typified by the MacMichael viscometer, gives the viscosity in arbitrary units. Smith (59) gives a critical discussion of viscometry from the technological point of view. In agar manufacture, it may be desired to determine the viscosity of liquors during extraction, and a rapid method is desired. As sludge is present, capillary viscometers are useless, and the MacMichael type is quite useful for this work. In the research on the agars from various seaweeds Ostwald viscometers were used as, at the time, they were the only instruments available, and they were considered to be accurate enough for the purpose. The errors of such instruments are known, but it has been found in practice that the most difficult part of the determination is to obtain uniform sols. This is because the history of a sol affects its viscosity, e.g. reheating a sol alters its viscosity, and even slight differences in the time of heating or of pressure during the preparation of a sol will give a difference in the readings. The standard method was to prepare a 0.25 per cent. sol by soaking the agar overnight in the requisite quantity of water, heating to 240°F. for 20 minutes, cooling to 158°F. and keeping the sol at that temperature until required. The viscometers were standardized against distilled water.

3. Elasticity.

Elasticity was measured by fitting a pointer to the arm of the balance used to test gel strength. The pointer moved along a scale calibrated in arbitrary units and the elasticity was measured by adding a known weight to the penetrometer and noting the movement of the pointer. On removal of the weight the final position of the pointer was also recorded, e.g. 5 g. moved the pointer through 10 divisions and on removal of the 5 g. the pointer returned through 8 divisions.

4. Setting Point.

Setting point was calculated by allowing a bubble of air to escape at regular intervals of approximately 1 second from a capillary into an agar sol. The gel strength and viscosity of the agar were determined and the percentage of agar adjusted accordingly, but was normally between 1 and 2 per cent. Within this range the change of setting point with concentration is unimportant. The setting point was taken as the temperature at which the bubble commenced to "hang" in the agar, failing to rise immediately and directly to the surface. This method proved adequate for the determinations. "Melting point" can also be determined by means of this apparatus (Fig. 1).

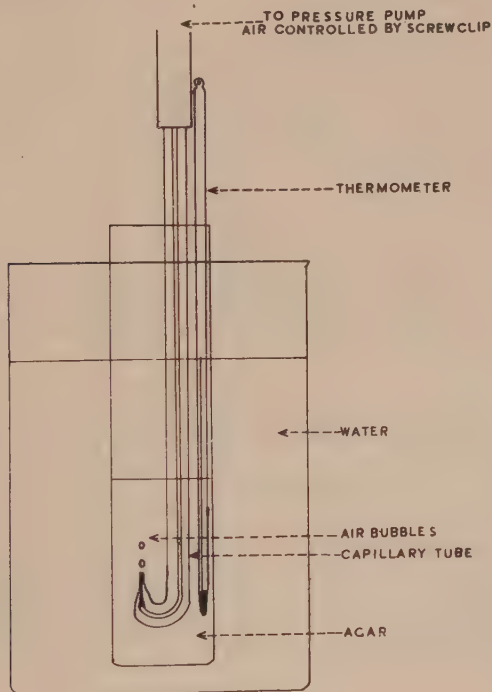


Fig. 1 - Sketch of setting and melting point testing arrangement for agar. The drop in temperature is regulated to 1° per minute and the setting point is taken as the temperature at which the bubbles no longer rise vertically, i.e. they "hang" in the agar.

VI. THE SOURCES OF AGAR.

Agar is derived from certain members of the red algae or Rhodophyceae and forms a constituent of the cell wall. Not all the Rhodophyceae yield an agar, though some give an agaroid.

In Japan the *Gelidium corneum* group are used, and *Camphylaphora hypnaeoides*, *Gracilaria confervoides* etc. are blended with it. The Russians use *Ahnfeltia* and make agaroids of the Irish moss type from *Iridaea* and *Phyllophora*. America uses *Gelidium cartilagineum*, and New Zealand, *Pterocladia lucida*.

Most of these weeds grow on rocky ledges and are difficult to harvest, requiring calm weather and low tides, hand picking or the use of diving gear. These impose severe practical limitations on the industry in America and New Zealand. *Gracilaria confervoides*, however, is found on sandy bottom in many of the estuaries of eastern Australia, and in the Swan River in Western Australia. For this reason it will be possible to obtain a quantity of raw material for agar manufacture at reasonable cost and largely independent of tides and weather conditions. Mechanical harvesting will cheapen the cost of the raw material and thus assist Australia to market agar at competitive prices.

The principal estuaries where *Gracilaria* occurs in quantity are Moreton Bay, Botany Bay, and Middle Harbour, Lake Woolaweyah (Big Lake) on the Clarence River, with smaller growths at Bateman's Bay, Shaw's Bay, Ballina, and a lagoon at Barney's Point (Tweed River) (Fig. 2). The areas of *Gracilaria* in the Clarence River Broadwater and the Swan River are extensive but less suitable for manufacture.

Close observation has been made of the beds at Middle Harbour and Botany Bay during the last two years, and there is no evidence that harvesting has had an adverse effect on the beds. There are many reasons why the weed should stand up well to cropping. These points are discussed in another paper now in press.

Experiments show that it is possible to grow *Gracilaria* on artificial cultch by planting rock or fibro-cement in suitable places at the right time. The seaweed can also be grown by wrapping wisps round fibro-cement or rock and embedding it in the sand.

With the exception of Middle Harbour, the seaweed beds have adjacent to them ample drying grounds. At Middle Harbour the local Council has aided the industry by allowing the use of parklands for drying the seaweed; otherwise it would be necessary to cart the wet weed to other areas to dry it.

Bleaching the weed is accomplished if desired by a continuation of the drying process, sunlight, moisture, and carbon dioxide being essential. The writer has found that bleaching the raw weed reduces the yield of agar and also the quality, but it has little effect on colour.

It is inadvisable to remove too much of the salt from the raw weed as salt keeps the weed reasonably moist during storage and prevents acid fermentation. Bleached weed

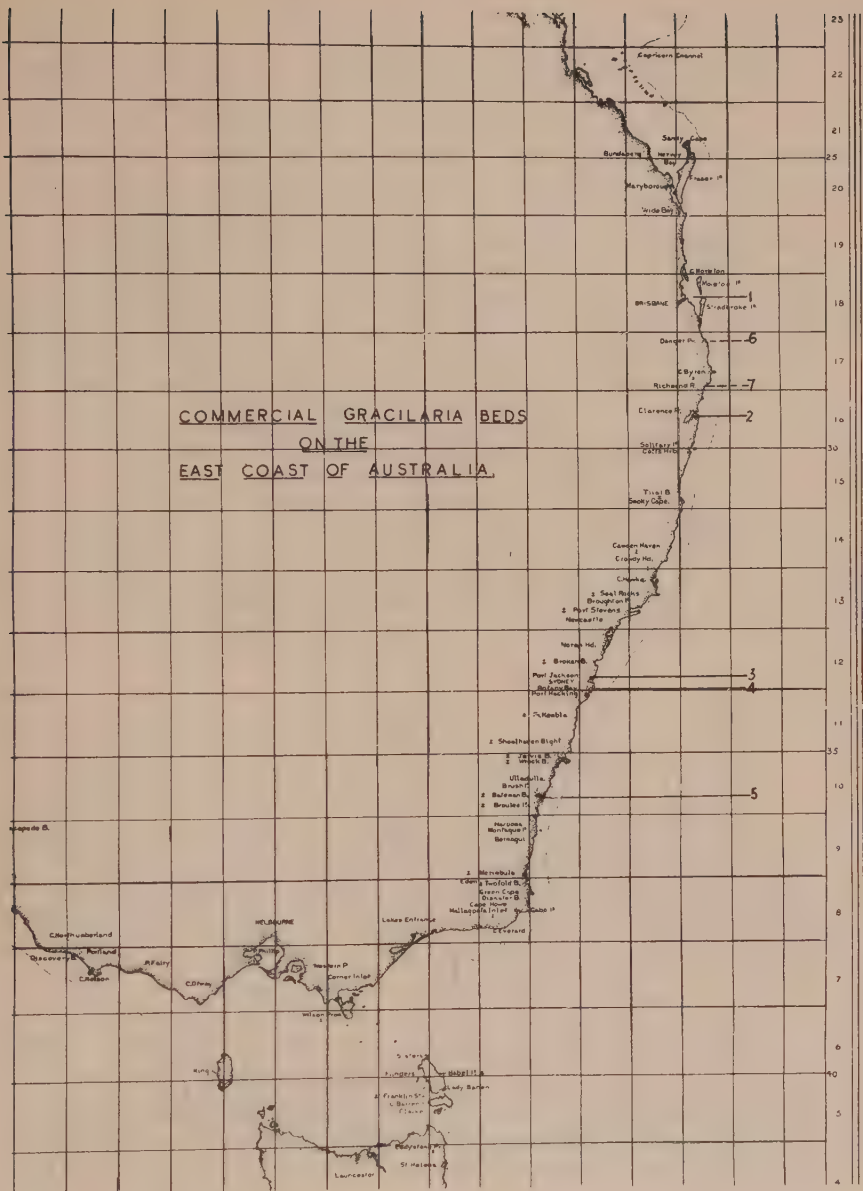


Fig. 2 - Map of South Eastern Australia showing locations of main Gracilaria beds. The dotted lines indicate small beds of good quality seaweed.

1. Moreton Bay.
2. Woolaweyah.
3. Middle Harbour.
4. Botany Bay.
5. Bateman's Bay.
6. Barney's Point Lagoon.
7. Shaw's Bay, Ballina.

from which salt has been removed powders in dry hot weather and is much more inflammable than the more natural weed.

Unbleached weed can be kept without appreciable deterioration for 18 months to 2 years and is easy to store in a dry place. In hundredweight bales it is easy to handle in transit and in storage (Plate 1, Fig. 2).

Eucheuma species are used for the manufacture of agar in Western Australia. This was suggested by the writer, and a small but very useful industry has arisen there. The weed is washed ashore at times in large quantities in the Abrolhos-Dongarra region though it appears to occur as far south as Bunbury. It grows on limestone ledges but extensive beds have not been located, although such beds must be present at least in the Abrolhos-Dongarra region.

Eucheuma is collected from the beaches, dried and bagged and is sold by the pound.

Owing to the entirely different techniques required for harvesting seaweed and manufacturing agar, it is suggested that these be regarded as separate industries. Supervision of the quality of the raw material can be exercised by the manufacturers and suggestions made to the harvesters for improvements.

VII. MANUFACTURING PROCESSES IN AUSTRALIA.

The following section includes the research carried out in this laboratory. The work was done using *Gracilaria confervoides*, except where otherwise stated. Commercial tests have been carried out in conjunction with various firms, but some of these results cannot be included. It has been found that the commercial procedures adopted usually follow those devised by this Division.

1. Treatment of the Weed Prior to Extraction.

It is customary in other countries to wash the seaweed prior to extraction, usually for about 24 hours. The following results (Table 5) show that with *Gracilaria*, washing the weed for any length of time decreases both yield and gel strength. Apparently this is due to the removal of the water-soluble fraction mentioned by Lipatov and Morozov.

TABLE 5 - Washed versus Unwashed Seaweed.

Sample	Crude agar	Fine agar	Gel strength fine agar
	%	%	g.
Washed	23.00	20.2	16.14
Unwashed	55.2	28.5	44.14

Crude agar is filtered liquor evaporated to dryness, fine agar has been purified by freezing and thawing.

These results are typical and were taken at random from

a number of tests. In the light of these tests it is recommended that the raw weed be given only sufficient washing to remove sand, mud, or other material which is likely to interfere with the boiling or filtration.

It was found, when translating laboratory experiments to a commercial scale, that the weed acted as an insulating material, and cold spots developed in the boiling vats. This was overcome at the time by mincing the weed, but a series of experiments has shown that the yield is reduced by mincing and the relative viscosity increased (7 average 1.6 for unminced against 2.4 for minced weed), although the gel strength is unimpaired. This was irrespective of the source of the weed, a number of samples being tested, the differences being significant in each case. An average of the results for a single batch of weed gave 13.75 per cent. yield of fine agar for minced and 23.5 per cent. for unminced weed. The reason for this is not clear. Duplicate samples of bleached weed were minced and boiled for 1/4, 1/2, 1, 2, and 3 hours, with unminced weed as controls, heated for similar periods. With minced weed, the greatest yield was obtained in 1½ hours, after which it fell steadily. The unminced weed gave a maximum yield after 3 hours boiling. The maximum yields were respectively, 25.5 and 31.3 per cent. These results were repeated on several occasions. The fact that the yield from unminced weed is still greater than that from minced weed shows that some factor other than time must also be operating.

It is now considered preferable to avoid cold spots in the boiling vats by vigorous stirring either mechanically or by live steam jets or both. These observations have been confirmed in commercial practice.

Bleaching of the weed prior to boiling is not recommended by the writer. Sun bleaching has already been discussed. Chemical bleaching at this stage can be obtained by the use of chlorine. Table 6 shows the most favourable result achieved, but it was not found possible to obtain uniformity.

TABLE 6 - Tests of Chlorine as a Bleach.

Chlorine bleach. Removed with thiosulphate and acidified with sulphur dioxide. No carbon bleaching.			Acid sodium phosphate prep. No carbon bleaching.		
Yield	Gel Strength	Viscosity	Yield	Gel Strength	Viscosity
% 20.0	65.4	1.8	% 26.4	39.2	1.8
The yield is appreciably lowered in the case of the chlorine bleach though in this experiment the gel strength was increased.					

2. Extraction of Agar from the Weed.

In this section, the following factors must be considered: temperature and pressure, time of extraction, pH of extracting liquor, buffering effects, ratio of weed to liquor.

(1) Temperature and Pressure. - Samples of weed from Botany Bay were tested according to the Laboratory routine, i.e. 4 per cent. weed extracted for 2 hours at pH 6.5,

filtered, frozen, thawed, and dried. Extraction temperatures were 95°, 103°, 109°, 115°, and 121°C. The results are given in Table 7.

TABLE 7 - Tests of Effects of Pressure and Temperature of Boiling.

Pressure	Temperature	Extraction	Gel Strength	Viscosity
	°C.	%		
atm.	95	30	47.6	1.4
2½ lb.	103	18	47.6	1.5
5 lb.	109	23.3	23.8	1.8
10 lb.	115	28.5	11.9	1.8
15 lb.	121	28.5	11.9	1.9

It will be seen that the percentage extraction was greatest with live steam at atmospheric pressure. Further, as the temperature and pressure increased, the gel strength decreased while viscosity increased. Korentzvit (31) advises using open vats and this seems advisable here. It was previously found that the yield using live steam at atmospheric pressure was much greater than that using a steam-heated or gas-heated pan.

(ii) Time of Extraction. - It has been found that the optimum time of extraction varies with the sample of weed under test. Samples from the same area dried by different people have given optimum extraction times of 1/2 hour to 4 hours. With efficient agitation the boiling takes 2-2½ hours in commercial practice and this seems the optimum time in the laboratory also.

No specific test has been found adequate to determine the boiling time of a cook. Specific gravity does not alter significantly, gel strength appears to remain significantly constant in constant dilution, though figures obtained immediately on cooling have been found unreliable: viscosity appears to increase steadily and then diminish after what is regarded as the optimum boiling time, and the determination of total solids supports the viscosity determinations. Practical experience possibly checked by tests on a Macmichael viscometer will be the most reliable means of deciding cooking times.

Table 8 gives 3 typical series of results of boiling time experiments.

TABLE 8 - Results of Tests to Determine the Optimum Boiling Time.

Experiment	Time	Agar	Gel Strength	Relative viscosity (average)
	hours	%		
I	1	26.7	53.0	1.4
	2	27.5	50.0	1.4
	3	28.2	58.2	1.5
	4	27.2	43.2	
	5	28.4	51.7	
	6	24.7	62.2	
II	1	28.5	34.4	1.4
	2	32.75	34.0	1.7
	3	29.5	40.0	1.7
	4	21.7	48.0	1.6
III	1	25.75	27.4	1.6
	2	27.25	33.1	1.5
	3	31.25	39.3	1.3
	4	27.25	38.1	1.5

In I the maximum effective extraction is reached in 3 hours, though there was no appreciable falling off in yield till after 5 hours. In II the maximum was reached in 2 hours and in III in 3 hours, and in each case the yield decreased. At times (see tests on minced and unminced weed) the maximum yield is reached in $\frac{1}{2}$ to 1 hour, and it is considered that with adequate agitation 2 hours is the maximum time for commercial practice. Owing to the variation between different samples, it is recommended that the boiling time be left to the discretion of the foreman or man in charge of the extraction; his judgment will give rather variable results, but if supplemented by viscosity tests will give an indication of the length of cook.

Two successive extractions of *Gracilaria* in this laboratory gave, for example, a yield of 30 per cent., whilst a counter-current extraction, using the liquor from a second boiling to extract a sample of fresh weed gave a yield of 40 per cent. One manufacturer working on a small scale obtains a 50 per cent. yield by counter-current extraction. In practice it is recommended that a short second extraction of 30-60 minutes be given, using water as the extracting liquid. The vat for the second extraction could be fitted with a false bottom overlaid by a rough filter material, e.g. hessian or coir matting, and the liquor run by gravity into the main extraction vat, filtration being by gravity. Counter-current boiling is used in California and at Vladivostok but Korentzvit finds it inadvisable for *Phyllophora* agar as it results in hydrolysis and decreased yields.

(iii) pH of Extraction. - In Japan, California, and New Zealand, the extraction is carried out in a solution on the acid side of neutrality. In Russia, *Ahnfeltia plicata* agar is extracted in alkaline solution.

A series of studies on *Gracilaria confervoides* from different sources show that there is no significant difference in yield with agar boiled at pH 6.5 and pH 10 but there is a lower yield between pH 7 and pH 8.

Below pH 5 the yield falls off rapidly to zero owing to acid hydrolysis. At low temperatures, the pH can be reduced to pH 2 but at boiling temperatures it is unwise to go below pH 5. Earlier work indicated that the optimum pH was between 5 and 6 but it may be desirable to boil at pH 9-10 in order to avoid the action of the acid liquor on the vats. If acid extraction is used, wood, stainless steel, monel metal, or porcelain lined vats are advisable. In commercial practice it has been found that filtration is easiest at pH 5-6.5 and this is an additional reason for acid extraction.

(iv) Buffering. - Normally *Gracilaria* liquor buffers automatically between pH 6.5 and pH 8, but weed from muddy areas may buffer in the vicinity of pH 9. It is found that *Gracilaria* which is grown on a muddy substrate, e.g. weed from Yamba or Moreton Bay, requires acid extraction. Until this was realised it was found impossible to get reasonable yields from such weeds. Now, the pH is adjusted to 6.5 using acid sodium phosphate, and excellent results are obtained. The use of this buffer substance results in a certain amount of flocculation, during which much of the pigment is removed from the liquor. This in turn makes the use of a chlorine bleach unnecessary, and simplifies the extraction. In fact, using unbleached weed, boiling with acid sodium phosphate, followed by a single filtration, a

colorless agar has been made in this laboratory from *Gracilaria confervoides*. The yield with single extraction was 31.4 per cent. and the gel strength 46 compared with 82 for New Zealand agar and 34 for Japanese.

The viscosity is consistently lowest in agars prepared by acid boiling.

In order that mild steel and other non-resistant metals may be used as much as possible, it is suggested that the liquor be made alkaline after filtration.

(v) Ratio of Weed to Liquor in Extraction. - A series of experiments has shown that the yield of agar tends to increase as weed/water ratio decreases, other things being equal. It is found, however, that unless the percentage of agar is high enough to give a firm continuous sponge after freezing, it is impossible to retain all the extracted agar after thawing. Above 4 per cent. weed/water the liquor becomes difficult to filter, and below 4 per cent. the thawed gel is not continuous. Therefore 4 per cent. is recommended as the standard concentration of weed, to give reasonable filtration, and a reasonably firm sponge if vacuum concentration is not to be used. The use of counter-current extraction will overcome these problems though it may be necessary to reduce the quantity of raw weed used,

(vi) Suggestions for Boiling Plant. - It is suggested that the weed be washed rapidly in a tumbler to remove sand and grit, but not so as to lower the agar yield. It should then be transferred to the boiling vat. This vat can be constructed of wood, manganese bronze, stainless steel, or monel metal and should be heated by live steam jets arranged in such a way as to give maximum circulation in the vat. Steam jackets may be used for auxiliary heating (Plate 2). The extraction temperature is about 95°C. The pH is best adjusted between 6.0 and 6.5 with acid sodium phosphate (1 part in 1200 approx.). Boiling time will depend on the weed used.

3. Filtration or Separation.

The commercial filtration of any colloid is not an easy problem but it is one for an engineer rather than a chemist. A few points however are important.

(a) The liquor must be filtered hot. *Gracilaria* agar sets between 45° and 50°C. at atmospheric pressure, and the viscosity and setting temperature increase with the pressure. For this reason it is advisable to keep the temperature of filtration as high as possible and the pressure as low as possible.

(b) The best method is to use suction instead of pressure to force the liquor through the bed (Plate 3, Fig. 1). A further advantage in the use of suction is that the pumps do not come in contact with the unfiltered liquor and are not subjected to abrasion by sand which always occurs mixed with the seaweed to some extent.

(c) A two-stage filtration is best, the first to remove the sludge and the second with filter aid and activated carbon to clarify the liquor.

(d) The first stage can be effected by (i) a false-bottomed vat with a coarse filter material such as canvas or coir matting on the false bottom; (ii) a steam-heated bag filter

(this has been used very successfully); (iii) centrifugation through a basket centrifuge (hydroextractor) using a canvas cloth inside the basket (this is also very satisfactory).

(e) Partial sedimentation is encouraged by using acid sodium phosphate. Experiments have shown that acid solutions give most rapid sedimentation. This is aided by the presence of phosphates which increase flocculation and aid the removal of colour with the floccules. By using horizontal filters and allowing some sedimentation to occur, the solids form a satisfactory filter bed and remove the need for a filter aid.

(f) Filtration through fine cloth filters using filter aid and usually charcoal is the usual method of clarification. The Sharples super-centrifuge has been found by the writer to be excellent for the purpose. Another method which has been successfully adopted in this laboratory is the use of a steam-heated carbon column. This has actually given the clearest product of all, and the resulting gel is colorless and transparent. Silica gel has been used in place of carbon, but was not satisfactory for decolorization.

Sedimentation is not sufficiently rapid or complete to allow the use of a subsider or thickener. This has been tried on the laboratory scale and various flocculants used, e.g. alum, trichloroacetic acid, glue, aluminium hydroxide, but none were adequate for commercial use unless the pH was kept well below 6. At this pH, acid-resistant metals are necessary and hydrolysis is likely to occur.

(g) Wooden filters either of the leaf type or of the plate and frame type can be used. Teak proved satisfactory in the laboratory but other hardwoods would no doubt be equally useful. A battery of suction filters is preferable to a large filter press as less time is lost in changing cloths. Horizontal filter beds are preferable (see above), (Plate 3, Fig. 1).

The use of activated carbon to clarify the agar is commercial practice in Russia and America, and now in Australia. Suitable grades of carbon for press filtration and for carbon columns are manufactured locally.

4. Concentration.

It is possible to concentrate agar to a limited extent *in vacuo* - say up to 3 or possibly 4 per cent., provided that means are taken to obviate frothing. Calandria type vacuum pans or Kestner type pans are suitable. Temperatures should be kept as low as possible to avoid excessive hydrolysis. Frothing should not be excessive at 10 inches of vacuum.

5. Purification.

Even when filtered with activated carbon and filter aid, agar contains impurities of a type which reduce the gelling power. Three methods of purification are known - freezing and thawing, alcoholic precipitation, precipitation with electrolytes, e.g. sodium sulphate. The two latter methods (see Sect. IV, 1, Properties of Agar) are those usually used for flocculating colloids. In each case the agar is purified, but both are inferior to the freezing and thawing process which is adopted by every agar manufacturer. From the fact that ordinary dialysis and flocculation methods remove many impurities but do not increase the gel strength to nearly the same extent as freezing, the writer concludes that there

is probably an alteration of structure during the freezing-thawing process. The writer has found that the gel strength is increased 5 to 20 times by freezing.

Because of the expense of refrigeration, and of the special difficulties in the way of providing and maintaining refrigeration in war-time, a number of attempts have been made to do without the purification of agar by freezing which is an inherent part of the process in other countries. Russian patents exist for the precipitation of agar by sodium sulphate, using the results of Buchner and Kleijn (5), and there are American patents for precipitation by alcohol, notably isopropyl alcohol (see below). The sodium sulphate method was tried but gave very poor results with a low yield and gel strength. One difficulty was the necessity for continued washing to remove the salt from the agar. Alcohol provides a useful method for precipitating agar quantitatively from solution (29,30) but as 4 to 5 volumes of alcohol are required for each volume of agar solution, the method is too expensive and would require too much space for commercial adoption. We are left once again with the freezing method, but it was thought that this might be eliminated by careful filtration. Accordingly, a sol was prepared in the usual way, and filtered through cotton wool and allowed to remain overnight in contact with activated carbon and diatomaceous earth. It filtered through paper giving a colorless liquor, and portion was frozen while the rest was dried at 40°C. The frozen gel was also dried at 40°C. and both were used to make up 2 per cent. gels and were analysed for moisture and ash. The following results (Table 9) only confirm previous observations.

TABLE 9 - Comparison of Frozen and Unfrozen Agar.

	Frozen	Unfrozen
Gel strength	80 g.	5 g.
Moisture	9.5%	9.6%
Ash	6.7%	12.1%
Nitrogen	0.53%	1.1%
Sulphur	0.45%	0.49%

Dialysis with and without freezing and thawing have also been tried. The results follow in Table 10.

TABLE 10 - Results of Experiments in Dialysis and Freezing.

	Frozen	Frozen		Not frozen	
		Dialysed 4 days	Dialysed 7 days	Dialysed 4 days	Dialysed 7 days
Yield	10.9	10.5	5.0	11.2	12.1
Gel strength	64.6 g.	50.2 g.	59.5 g.	*	*
Viscosity	1.8			1.4	1.4

* Too sloppy to test.

Good results from dialysis have been claimed in Australia but the writer's experiments completely fail to confirm this either on laboratory preparations or on commercially prepared dialysed agar samples. The latter were invariably of poor quality.

6. Effects of Freezing.

The mechanism of purification by freezing is not very clear. It would seem that impurities are squeezed out of the gel by a process closely resembling syneresis, in which Rossi and Marescotti (54) note that the syneresis liquor contains a higher percentage of salts than the retained liquor (see above). The facts that washing the seaweed reduces yield and gel strength and washing the dried agar itself removes a stabilizing factor of the gel have to be taken into consideration. As, after freezing, gel strength is increased and only impurities are removed either in thawing or in subsequent washing, the surface of the gel must act as a dialysing membrane. Some of the intermicellar liquid with electrolytes, pigments, and probably degraded proteins, apparently freezes independently of the gel and is not taken up on thawing. Thawed gels, however, will swell if left in contact with water but much of the water can be expressed by pressure. The inorganic and organic material, composing the water-soluble fraction of agar of Lipatov and Morozov (36,37) which is water-soluble when dry, is apparently firmly combined in the gel micellae and is therefore not extruded. Gelation curves were plotted of a 3.0 per cent. agar solution diluted with 10 per cent. of the following:-

- (i) Water in which *Gracilaria* had been soaked for 24 hours.
- (ii) Distilled water.
- (iii) Extruded water from thawed agar.

These liquors were then diluted to various strengths between 0.7 and 2.2 per cent. and curves of gel strength plotted. It could be shown that neither the wash water (i) nor thawed water (iii) had any stabilizing or retarding effect on the gel, and so the theory that the purification is by osmosis receives strong support.

The amount of water frozen out of a given gel did not seem to be constant for any set of conditions, but usually 60 to 80 per cent. of the original water present in the gel. Vasil'ev and Guenis (66) state that the quantity of water eliminated from agar gels by freezing is independent of the method of freezing and recommend brine freezing at -10°C ., rapid thawing at 40°C ., and centrifugation. The initial concentration affects only feebly the duration of freezing. As well as a purification, there is also a considerable concentration of the gel due to freezing and thawing. Subsequent washing of the gel seems to remove further impurities.

In purification by freezing, the factors studied were:- initial concentration of gel, temperature of freezing, temperature of thawing, and pH of liquor.

(i) Relation between Concentration of Gel and Yield of Agar.- Experiments with different concentrations of gel showed that the recovery of agar increases with the dilution of the gel until the gel becomes too weak to

allow of proper separation, i.e. loses coherence. This occurs when the concentration of agar falls much below 0.75 per cent. The statement of Zhelezkov (68) that the percentage of water extracted is less with increased concentration was not confirmed. Usually between 50 and 70 per cent. of the original water was removed, but this appeared to be related to time of thawing and time during which thawed water was left in contact with the gel.

(ii) Optimum Freezing Temperature.— Temperatures between 32° and 5° $^{\circ}$ F. were used. The optimum was found to be between 10 and 21° $^{\circ}$ F. probably about 17° $^{\circ}$ F. This agrees with the Russian results (29). There does not seem to be homogeneous freezing at 5° $^{\circ}$ F. although some industrial experiments in Australia and some Russian results (14) suggest this. The discrepancy may be due to the use in this laboratory of air freezing without circulation, while in the other tests brine was used. It has been suggested that the size of the ice crystals (i.e. rate of freezing) is the important factor. Larger crystals are preferable.

(iii) Relation between pH of Freezing and Yield.— Experiments with pH were indecisive, i.e. there was no significant difference in the results obtained in a large number of tests. The conclusion therefore is that the pH of freezing is unimportant in respect to yield and gel strength. It is, however, preferable to freeze on the alkaline side, as non-resistant metals can then be used (Plate 3, Fig. 2).

(iv) Thawing.— For rapid thawing it is preferable to thaw agar in water rather than by draining. Tests at temperatures from 40° to 20°C. showed that the greatest yield and highest gel strength were obtained at 20°C. though there was no marked difference at temperatures up to 35°C. It is doubtful whether it would be economical to thaw at temperatures below 20°C. The residual water should be expressed as soon as thawing is complete owing to the formation of a hard surface film that seriously impedes drying. Centrifugation is best for water removal.

7. Drying.

Laboratory tests showed that:—

- (a) The rate of drying agar gels is constant for a given thickness of gel but the gradient of the drying curves increases with thickness of agar.
- (b) With agar gels of concentrations between 1.04 and 1.92 per cent. the rate of drying is independent of concentration.
- (c) During the major part of the drying, the rate of drying is constant. It increases with increased temperature but the increase appears to diminish with each 5° rise of temperature. 35–40°C. is the optimum temperature for drying. Above 50°C. marked discolouring occurs. One of the chief difficulties in drying is the formation of a surface-film.

(1) Methods of Drying.

- (a) Spray drying.— A primitive spray dryer was rigged and this showed that agar could be successfully spray-dried.
- (b) Drum drying.— A drum dryer was not available. Successful films of agar were dried on the surface of a steam-

jacketed pan. Drum drying (in *vacuo*) is used in Russia.

(c) Tunnel drying.— This was used as a routine procedure in the laboratory and in pilot plant tests at Cronulla. Tunnel drying is slow and there is a tendency to discoloration of the agar.

(d) Infra-red light.— (Plate 4, Fig. 1). This was the most successful method used. The agar was invariably lighter in color and the time of drying was shortened by 12 hours. In the tunnel drier 500-2000 watts were used and in the infra-red battery 1200 watts. The latter dried agar 1 inch thick in 4 hours. As the optimum temperature of drying decreases with decreased moisture, heating can be stronger with wet than with partly dry agar. Using infra-red lamps and a moving belt, full advantage can be taken of this by setting the lamps close to the wet and far away from the dry end of the belt.

(ii) Drying Surfaces.— It was found difficult to obtain a satisfactory material on which to place the agar for drying. The drying gel tends to adhere to metals and to glass and to absorb colored ions. Phosphor bronze wire gave blue-green agar and a slight flaw in tinned iron wire colored the agar dark brown for 3-6 inches around it; cheesecloth was the most satisfactory material, though galvanized wire trays can be used if the pH of the agar is above 7.

A happy solution of this problem is due to the Karna Vita staff with whom the writer has lately been in close collaboration. They showed that agar could be rapidly dried by immersing the thawed material in alcohol. The alcohol removes much of the color and makes for very rapid drying. In addition, it gives a substance which no longer adheres to ordinary surfaces. The Company has very generously given the writer permission to publish this method.

8. Suggested Methods of Manufacture (See Fig. 3).

The dried weed is received at the factory, and tested for moisture, inorganic matter (sand, shell etc.), and foreign weeds. Suggested standards are:— moisture 18-20 per cent., sand etc. 10 per cent., foreign weeds 2.5 per cent. (as foreign weeds do not prevent the extraction of agar, this percentage may be raised if desired). The weed is then placed in a dry airy store. If the weed is too dry (10 per cent. or less moisture) it becomes brittle and forms dust, which on subsequent washing will clog the wastes. Salt left in the weed helps to keep it from becoming too dry and to render it non-inflammable. On one occasion a load of bleached weed caught fire, but whether this was due to spontaneous combustion or to human agency is not certain.

On removal from store the seaweed is washed for a short time in a tumbler or dolly, drained, and placed in the boiling vat. As it is an advantage to keep the pH acid during boiling (at or below 6.5 with acid sodium phosphate) the boiling vat should be of a non-corrosive material. Stainless steel, aluminium, or monel metal are satisfactory and wood might be used. Live steam is used for heating and agitation. The concentration of weed to water is 4 per cent. It is preferable to boil the weed twice and to use the liquor from the second boiling as mother liquor for boiling the raw weed. This increases the yield by about 20 per cent. and removes a great deal of already extracted agar from the first residues. Times recommended are:— $\frac{1}{2}$ hour for second boiling, 1-3 hours for first boiling according to the rate of agitation and nature of the weed.

FLOW SHEET OF AGAR PLANT.

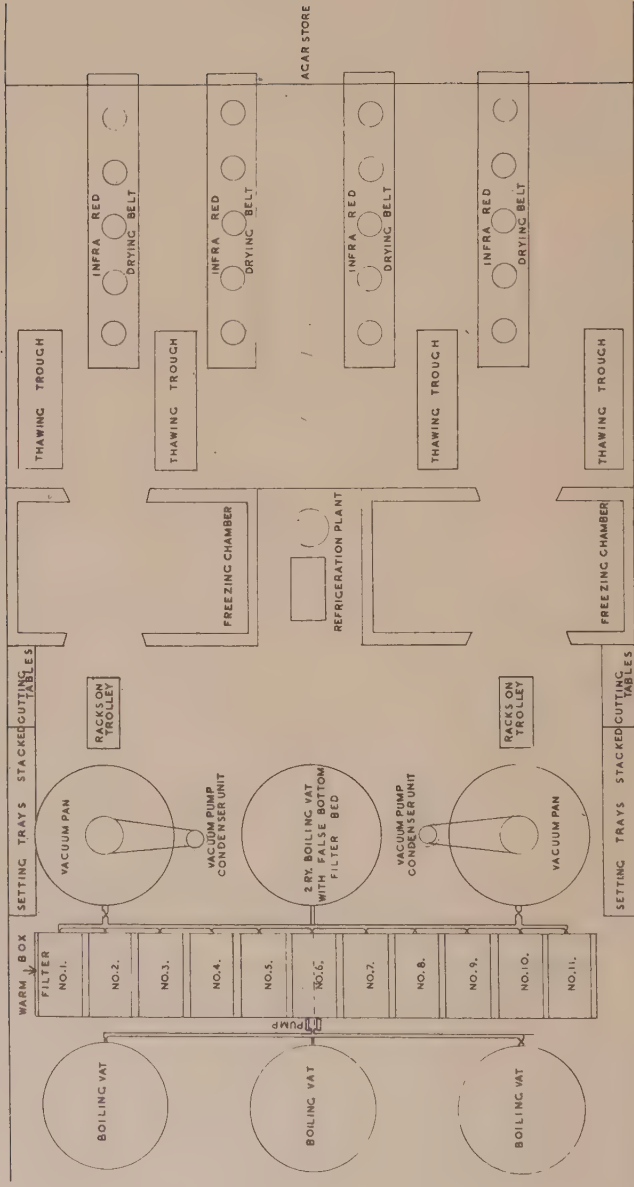


Fig. 3 - Flow sheet of suggested agar plant.

The boiling should continue until the first high viscosity has decreased, and the liquor should be filtered through a coarse canvas, either in a press, a filter bag, or a centrifuge. It should be run into a steam-heated vat, neutralized, and filter aid and activated carbon added if need be. Thence it passes into the second filter or super-centrifuge for clarification. Suction filters should be used to avoid sand in the pumps and to increase the amount and rate of filtration through a given surface. With their use a single filtration may suffice. The clear liquor is evaporated if desired and allowed to set in thin or shallow vessels, frozen at 15-20°C. and thawed out in running water or in air. The water can be used as a transporting agent as the agar-ice mixture floats. The gel is then centrifuged to remove excess water, washed in alcohol, and dried in a few minutes, preferably by batteries of infra-red lamps.

VIII. SUMMARY OF EXISTING PATENTS.

1. U.S. Patent No. 1,453,848, May 1, 1923, by C. Matsuoka, a Japanese subject. The seaweed (*Glotalpsittis*) is dried in the sun, bleached with 1 part of liquid chlorine to 9 parts of seaweed, and then treated with a 10 per cent. solution of sodium thiosulphate to render the seaweed odorless. The seaweed is then rinsed, boiled at over 100°C., and filtered through cloth. The jelly is allowed to form and then comminuted and frozen on trays, being finally dried in the sun or in a drier. The dry agar is cut into strips and washed in water for from 3 to 10 hours, preferably with 1 per cent. of sodium bisulphate. (Possibly sodium bisulphite is meant as bleaching is referred to).

Matsuoka then dips the re-dried agar sheets in sugar syrup and powders it. This of course does not give a pure agar, and the agar so prepared would not be acceptable for bacteriological or chemical purposes.

2. U.S. Patent No. 2,011,594, Aug. 20, 1935, by W. Seltzer. This is stated to apply to Irish moss, but would be equally applicable to agar. The Irish moss is added to 3 parts of ethyl alcohol to 1 part of water and then centrifuged. Isopropyl alcohol may also be used. The centrifuged mass is washed with the appropriate alcohol.

3. U.S. Patent No. 2,231,283, Feb. 11, 1941, by A. Pfister. This is also applied to Irish moss but could be applied to agar. The seaweed is extracted and filtered and the filtered product run into isopropyl alcohol. The resulting precipitate is centrifuged and pressed in an hydraulic press. A hammer mill may be used to facilitate subsequent drying which should take place at 50 to 60°C.

4. U.S. Patent No. 2,231,284, Feb. 11, 1931, by N. Blihovde. This patent is essentially similar to No. 3 but a special mixing valve is described.

5. U.S. Patent No. 1,712,785, May 14, 1929, by John Becker. In this process the weed is washed in 4 changes of water for 24 hours, boiled in 10 per cent. solution, and cooked under pressure. The extract is filtered with filter aid and activated carbon, and evaporated to a constant strength. The liquor is passed into a "congealer and sizer" to set it

and divide it into suitable sizes prior to freezing. It is frozen in ice cans in a brine bath and then the agar-ice is crushed and thawed in water. The thawed agar is introduced into a tower and blown upward in a current of warm air, into a hopper.

6. U.S. Patent No. 1,726,942, Sept. 3, 1929, by John Becker is for a "dewaterer", practically a rotary filter giving continuous removal of water from flaked agar.

7. U.S. Patent No. 2,200,774, May 14, 1940, by T.W. Halliday is to prepare flake agar, but as certain substances, e.g. psyllium seeds or paraffin, are used to lubricate the rollers, the resulting agar would not be pure.

IX. THE POSSIBILITIES OF THE AGAR INDUSTRY IN AUSTRALIA.

Agar of excellent quality, better in some respects than Japanese agar, can be produced in Australia from *Gracilaria confervoides* (Plate 4, Fig. 2). The better quality agar produced in this laboratory and commercially has been more than comparable with Japanese agar, the gel strength being up to 100 per cent. higher than Kobe No. 1. However, the viscosity and setting point are also higher. The viscosity is reduced by dialysis of the frozen agar and by bacterial maceration. Numerous experiments have been made in this laboratory to reduce the setting point but have not so far proved successful. It is assumed that viscosity (to some extent) and setting point are characteristics of the seaweed though viscosity does vary to some extent with the batch of weed used.

Bacteriological tests made by Dr. H.L. Jensen showed an excellent growth of haemophils and other pathogens, but some inhibition of growth with *Rhizobium* species. Blood plates were rather more difficult to pour, owing to the higher viscosity of *G. confervoides* agar. The greater toughness of Australian agar makes the streaking of plates much easier. The writer has found the latest preparation almost equal to *Pterocladia* agar in gel strength, clear and colorless, with a strong surface for streaking organisms. In making urea media, however, where the urea must be added below 50°C. great skill was necessary to avoid premature setting of the agar.

Meat canners consider the agar at least equal to Japanese agar and probably a little better, while confectioners found some difficulty with frothing of agar-sugar mixtures, though this was later overcome when the agar improved in purity. Some cordialmakers prefer *Gracilaria* to other agars on account of its higher viscosity. Australian agar is quite successful for dental use. It has been alleged that *Gracilaria* agar is grey coloured, and this is undesirable, but, with the correct type of activated carbon, glass-like, completely colorless and transparent agar can be produced. Summing up, a very high graded agar can be produced from *G. confervoides* if the necessary precautions are taken (Plate 4, Fig. 2). Greater experience in manufacture should improve the product, and it may be necessary to blend certain seaweeds for some purposes.

It is suggested that a powdered agar be prepared. This can be produced by drum drying, or spray drying, or by the powdering of sheet or strip agar. It will not be economical to produce strip agar similar to Japanese, and powdered agar has advantages over flake agar except for direct use as a laxative.

The costing of a new industry is a difficult matter. At present the raw *Gracilaria* is being harvested at £60 per ton but the cost might be reduced somewhat if unbleached seaweed were used. £60 per ton means £120 per ton for raw material in terms of 1 ton of agar on a basis of a 50 per cent yield. At a wholesale peace-time price of 3/- per lb. of £336 per ton, £216 is allowable for manufacture.

The plant could be mechanized and labour reduced to a minimum so that the principal costs would be:- Steam for heating the large quantity of water required in boiling 50 tons of water per ton of agar and in keeping the solution hot during evaporation and filtration; refrigeration to the extent of 50 tons per ton of agar, and the depreciation and interest on the relatively large capital involved. Peace-time production at such a price would require all capital expenditure to be written off, and a maximum efficiency in harvesting and manufacture to reduce costs to a minimum. At present, agar is 20/- stg. per lb. or \$4.25 in U.S.A. so that there is ample margin of profit. It is considered that the industry should continue as a peace-time one provided that capital is written down as rapidly as possible, even if agar returns to its pre-war minimum price. In poor seasons in Japan, agar rose to 5/- or 6/- per lb. which would give quite a reasonable margin. Actually, producing firms aim at a permanent industry in Australia.

The principles involved in agar production are simple, and, apart from the freezing, do not require expensive machinery. Suction filters, apart from expensive filters of the Oliver type, are not usual but suitable filters of cheap materials can be built up into batteries and, in one plant, have been so built. The most costly part of the manufacture is the large amount of water required in the preparation, approximately 45 tons per ton of agar. This water has to be boiled twice, passed through filters, frozen, and finally removed. Agar as finally prepared contains 16-23 per cent. water, so complete removal is not required. This is important, as the removal of the last traces of water greatly increase the cost of any process. However, the treatment of approximately 10,000 gallons of water per ton of agar or 500,000 gallons per annum for a 50 ton p.a. plant is actually the most costly part of agar production. The actual cost of such treatment depends on whether the steam used for heating and the refrigeration plant are required for agar alone. If exhaust steam from other processes is used and refrigeration is required for other purposes, the manufacture of agar becomes much more economical. In fact it is strongly recommended that in order to meet post-war competition, agar plants should be erected in conjunction with other industries, preferably chemical.

It appears from our collected information that the industry will naturally be centred at Sydney and Brisbane. Smaller plants may arise and work profitably in Western Australia and possibly in Tasmania. Of these, Queensland probably holds most promise, as the beds at Woolaweyah Lake and in Moreton Bay could be tapped, but much suitable weed is very close to Sydney.

From the studies made to date, it is believed that Australia can henceforward provide all her own needs of agar. Whether an export trade develops depends on the quantity of seaweed available, the cost of production, and the way in which the seaweed beds stand up to harvesting over a period of years. This means that studies on the seaweeds will need to be continued so as to insure that our resources will not be over-exploited. Latest surveys indicate that raw material will allow for an expansion of the industry far in excess of Australia's requirements, provided care is taken in supervising the harvest.

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APPENDIX I.METHODS OF PREPARATION OF AGAR IN USE OVERSEAS.

It is considered advisable to give a brief account of the agar industry in other countries as this must have a bearing on the future of the industry in Australia or on the Government's policy towards it.

The most primitive method is that used in Japan. A detailed account of the Japanese industry is given by Dickover (9). The *Gelidium* is collected, dried and baled and carried to the mountains where the farmers make agar as a winter occupation. The weed is pounded in a stone mortar, washed in running water, spread on mats and dried for 10 days or so. In this way the weed is bleached and dried. As soon as the weather becomes frosty, the seaweed is boiled in large kettles for about 25 hours, ladled out, and filtered through a hemp mesh bag to remove the fibre. The filtrate is crude agar. This is allowed to set in shallow wooden boxes, and to freeze for several nights. After this, it is cut to fit a wooden pump with a wire mesh at the lower end. A piston forces the agar through the wire which forms strips of agar $3/8$ inch square and 15 inches long. These are spread on mats and laid out in the weather for a fortnight or so, freezing each night and thawing each day. When dried, these strips are baled. This is the form in which agar is exported from Japan.

This procedure avoids the use of metals as far as possible.

The output obviously depends on the quantity of weed available, and on the severity of the winter. Big fluctuations in the annual production of agar in Japan and in the price thereof are usual. It is difficult to assess the state of the industry, but it seems probable that great expansion is unlikely for the following reasons:-

1. The production in favourable seasons seems fairly constant, and is not rising sharply, although the demand has increased in recent years.
2. Japan has been fostering her seaweed industries for many years, as they supply food to an overpopulated country. Possibilities of expansion must therefore have been carefully examined.
2. Pollution of waters by industrial wastes has been reducing the amount of raw material available. When this happens it is necessary to compromise between the two industries, and such compromise usually restricts the maritime one.

The American agar industry and its origin has been described by Robertson (52). Agar was first prepared by Japanese from *Gelidium cartilagineum* at Glendale in California but artificial freezing was found necessary, so the industry was reorganized, and a plant built at San Diego. There are at present five agar plants in California. The output has varied from about 40,000 lb. per annum to about 1,000 per annum, mainly owing to harvesting difficulties. Because of the extreme shortage of agar, strenuous efforts are being made to produce 200,000 lb. per annum. The weed is harvested by divers, who, according to Tseng (private communication) can collect as much as 1600 lb. of wet weed per day. Under

Australian conditions, the cost of the raw material collected in this way would be high, and the writer believes, uneconomical.

In the manufacturing process described by Robertson (U.S. Patent 1,712,785) the raw dried seaweed is washed for 24 hours in wooden vats with 6-hourly changes, and transferred in iron baskets to retorts where it is steam digested for 6 hours in a liquor from the partly digested weed of a previous batch. The ratio of weed to water is approximately 1-lb. per gallon. Each batch of weed is extracted three times, for six, eight, and twelve hours by countercurrent, i.e. the raw weed meets the most concentrated liquor. This liquor is clarified, treated with activated charcoal and a filter aid (Filter-Cel) and fed at 30 lb. pressure to a plate and frame press. When filtered, the agar is allowed to set (its concentration being 1-2 per cent.), fed to a shearing machine, and the fragments fed to ice cans, and frozen as in ordinary ice production. The frozen gel is crushed, thawed in a rotary washer, and centrifuged. The agar fragments are dried in a special type of aluminium dryer on the cyclone principle.

American agar is grey, has a higher gelling power, and absorbs more water than Japanese agar.

It is probable that many modifications are embodied in the new plants erected in California recently, but no information on this point is available. One would expect the use of stainless steel and other non-corrosive metals in the retorts, baskets etc. to improve the colour. Two paragraphs of Robertson's paper are worth quoting as they agree closely with the writer's observations.

"The elimination of the expensive freezing operation attracts interest, but so far without promise of commercial success. When the agar liquor is simply jellied and dried, a tough horny material is produced. It forms relatively large masses and is very difficult to crush or cut up. It is difficult to disperse again in hot water. Furthermore, if a simple evaporation method were adopted, it might be necessary to dialyse the particles for a long time to get the undesirable salts out of the mass. The use of glass or enamelled industrial ware would undoubtedly permit the production of pure white colourless agar if a real demand should come for such a material. Experimental batches of such character have been produced in the San Diego laboratory. Agar is so highly absorptive that the slightest discoloration is easily acquired and quite obvious to the eye, although the actual percentage of coloured impurity may be almost infinitesimal."

The Russian processes are in some respects an improvement on the American, but the differences between the *Phyllophora*, *Iridaea*, and *Ahnfeltia* agars of Russia, and *Gracilaria* agar of Australia, make different processes necessary. As it is possible that other types of weed may yet be used for agar in this country, it seems advisable to take note of the Russian work.

Korentzvit (31,32) describes the technology of the agar derived from *Phyllophora rubens*, which Kizevetter (29) classes as an agaroid. The first factory production in U.S.S.R. was at Odessa late in 1932. The alga is washed and then boiled in open vats at 95°C. Korentzvit finds that pressure, although it considerably accelerates agar production,

also increases hydrolysis and therefore decreases yield. The time was reduced from 7 hours with boiling water to 1 hour at 2 atmospheres. A preliminary treatment with sulphurous acid accelerates agar production but increases hydrolysis if pressure is used. Long heating causes hydrolysis and sulphurous acid has a beneficial effect. Korentzvit finds that separate extractions are preferable to countercurrent extraction, on account of hydrolysis. He further concludes that the capacity of the diffusion vessel should not exceed one cubic metre, but he is obviously using vessels in which there is no stirring action. Stirring should reduce the time of extraction very considerably. With open steam coils, and a stirring mechanism in the vat, size of vessel should not matter. Filtration is through linen and then through a filter press with activated carbon.

Finally, *Phyllophora* agar is allowed to set, cut into slabs, and dried, but bacterial contamination and high nitrogen content spoil the product, and Korentzvit recommends spray or drum drying. Apparently freezing is not used. This would account for the high nitrogen content.

Kizevetter (29), describes the production of agar-agar from *Ahnfeltia plicata*, and his treatment of the subject is exhaustive. It is advisable to give a full discussion of his work in order that the reader may note the similarities and differences between it and the process developed here for *Gracilaria*.

As agar from *Phyllophora rubens* was not suitable for all purposes, further researches were undertaken and, for a time, *Iridaea* was used. It was found, however, that *Ahnfeltia plicata* gave a very good quality agar and six fields with an estimated possible production of about 16,000 tons of dry weed, were located, while the nature of the beds allows rapid mechanical collection of the seaweed. This figure seems exceedingly high and would represent a possible 2,700 tons of agar, a quantity approximately equal to the total Japanese production. As this weed has also been found in the White Sea and is worked at Archangel we may expect a big increase in the world production of agar after the war. Only about 55 tons of agar and 200 tons of agaroids were produced in Russia in 1937, the agaroids being produced at Odessa. As Russia appears to be still short of agar, the possibilities of *Ahnfeltia* agar do not appear to have been realised.

The seaweed is collected on the beach after storms or by big trawls on the beds and is sun dried, being turned at frequent intervals to avoid rotting. Kizevetter assesses the quality of the raw material by determining the moisture (by drying at 105-110°C.), mechanical admixtures, and water-soluble substances. Washing, Kizevetter notes, does not affect the colour appreciably. *Ahnfeltia* should not be dried above 85° but 80°-85°C. has no deleterious effect. Bleaching the seaweed did not improve the quality of the agar, nor did the cutting up of the raw material improve the yield rate of digestion. The optimum pressure for boiling for 1 hour is 4-5 atmospheres with a working range of 2-4 atmospheres. Increased time of boiling increases yield up to an optimum and then decreases sharply. The optimum time decreases with pressure. Kizevetter considers it essential to adjust the pH on the alkaline side, and uses 8 per cent. lime in digestion. He found also that 10 to 12 per cent. of agar remains in the solid residue after the first boiling. The best results were obtained by boiling at 1 atmosphere for

6 hours, then 4 hours for the second extraction, and 2 hours for the third, or by boiling at 2 atmospheres for 2 hours, 1 hour, and 1 hour respectively, though the quality was admittedly lowered by pressure cooking. It would appear that live steam heating in open vessels might be preferable for extracting *Ahnafeltia* as it is for *Gracilaria*.

The digest can be decanted to remove grosser material, and finer material is removed by filtration through conical filters of cheap cotton material, but the temperature during filtration should not drop below 80°C. The more concentrated the solution, the higher the temperature. Kizevetter found that passing the liquor through an activated carbon column was not effective in decolorizing, but that adding activated carbon to the liquor and filtering with the addition of a filter aid produced a clear and colourless liquor.

The filtered liquor is allowed to set in water-cooled moulds. Kizevetter (29) and Vasil'ev and Guenis (66) recommend washing the gel to decolorize it and claim that it is cheap and effective. Fresh water must be used and the temperature kept below 10°C., preferably 3°-5°C.

The gel is then frozen; Kizevetter likens this to a process of irreversible coagulation, but it is, in fact, a process of dehydration without discharge, i.e. of syneresis. The intermicellar water is forced out and the process is irreversible so long as temperature conditions are maintained, but as the temperature is raised during thawing a proportion of the water can be re-absorbed. For this reason, the frozen and thawed agar behaves in most respects like a normal gel.

The freezing process should be slow, to avoid the formation of small ice crystals and, in extreme cases, homogeneous freezing of the gel. Quick freezing gives a poor concentration of the agar and consequent poor removal of impurities. Kizevetter states that the water used for thawing and washing should not be above 10°C. Concentration for freezing should not exceed 2 per cent. dry agar. Kizevetter suggests pressing the thawed agar. He finds that drying at high temperatures gives a more highly coloured product than slower drying at lower temperatures. The product dried at high temperatures swells less in water, and dissolves less readily. He considers that evaporation prior to freezing should be done a number of times at low temperatures.

Drying should be below 80°C. and a drum dryer is recommended, working *in vacuo*.

Kizevetter suggests the precipitation of agar by stirring a cooling sol (30). The writer has experimented with this process without success in the case of *Gracilaria* agar - tests by a colleague have also been unsuccessful.

This account of Kizevetter's researches, when compared with the method worked out independently for *Gracilaria* agar, shows how much the processes of manufacture and properties of agar agree. The most striking difference is the optimum pH, and this is very important in each case. There are some temperature differences also.

PLATE 1.



FIG. 1 (above).—Method of testing gel strength of agar.

FIG. 2 (below).—A hundredweight bale of *Gracilaria*. The weed in the foreground is bleached and that in the background unbleached.

PLATE 2.



Boiling vat used in pilot plant experiments. (By courtesy of Karna Vita Ltd.)

PLATE 3.

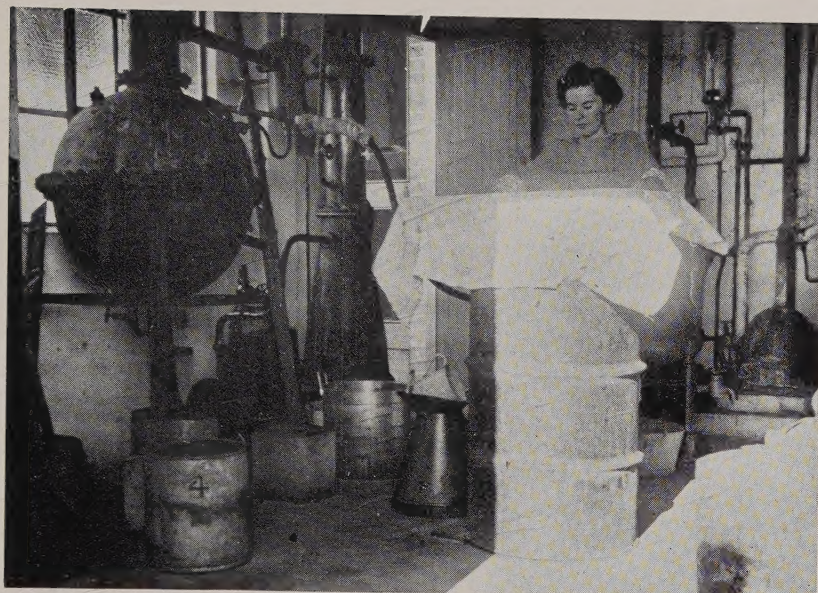


FIG. 1 (above).—Setting the filter cloths in the pilot plant. Note the horizontal filter bed, suction pipe and vacuum pan. The last mentioned is used as a suction reservoir at the same time concentrating the liquor. (By courtesy of Karna Vita Ltd.)

FIG. 2 (below).—Examining frozen agar. The agar shown is frozen by air blast, and is thawed and dried on the wire racks. It readily leaves the racks when treated in this way.

PLATE 4.

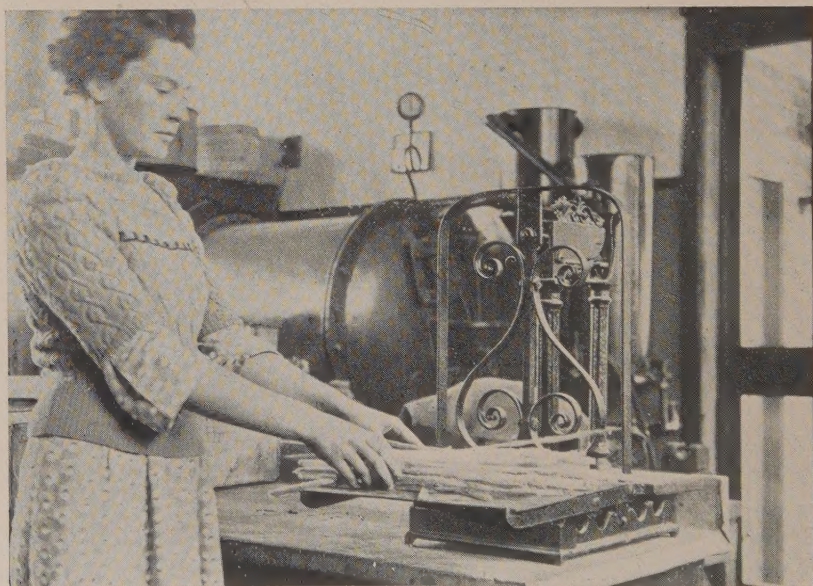
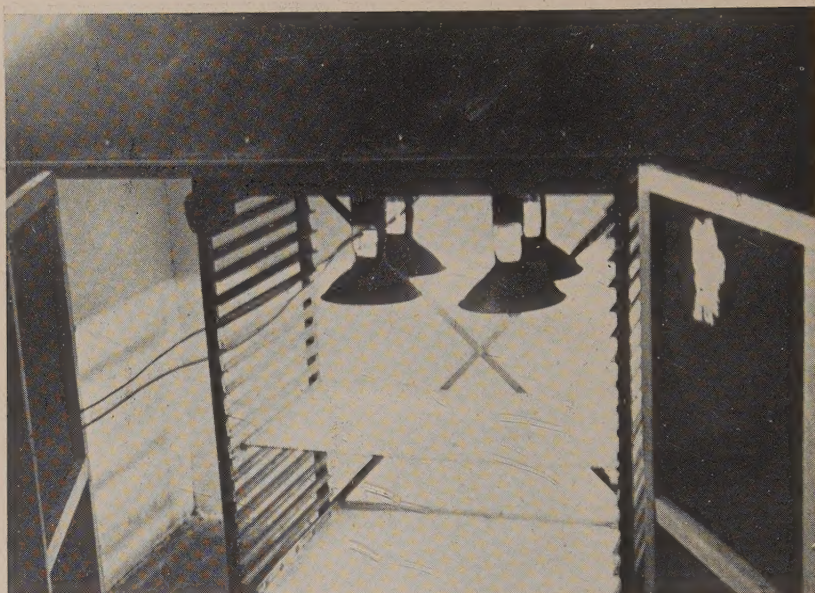


FIG. 1 (above).—Infra red lamps in use. Below the rack is a reflecting surface. The rack can be raised or lowered for convenience in pilot plant work.

FIG. 2 (below).—Weighing Australian agar. The product shown was prepared to resemble Japanese agar, except for the thicker strips produced.

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